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macrogonidium. *B*, a more advanced stage; the microgonidium has fastened itself directly on the body of the macrogonidium, and its substance begins to penetrate into the latter. In both individuals the nucleus has separated into small rounded fragments, and in the microgonidium are seen the two striated segments resulting from the division of its nucleolus. *C*, last stage of the conjugation. The microgonidium, completely void of its contents, remains attached to the body of the macrogonidium under the form of a minute hollow tube, which in the end drops away;—*ma*, macrogonidium; *mi*, microgonidium; *n*, nucleus; *nu*, nucleolus; *vc*, contractile vesicle. (From Balbiani.)

Fig. 4. *Stentor coerules* fixed upon a conferva filament, enlarged fifteen diameters. (From Balbiani.)

Fig. 5. *Stylonychia mytilus*, in position preliminary to copulation. The individuals are in contact by their ventral faces. (From Balbiani.)

MICROSCOPY.¹

Kultschitzky's² Methods of Staining the Central Nervous System.—The tissue, having been hardened in Müller's fluid, or preferably in Erlich's fluid, imbedded in celloidin, and sectioned, is allowed to remain in the stain from a few minutes to several hours. The first solution of hæmatoxylin is made as follows:

Boric acid (saturated sol.)	20 ccm.
Water (distilled)	80 ccm.
Hæmatoxylin	1 g.

The hæmatoxylin should be dissolved in a small quantity of alcohol, and then mixed with the boracic acid and water. The resulting fluid will at first be yellow, but in two or three weeks it will change to a dark yellowish red. *Before using, the solution should be acidulated with a small quantity of acetic acid*, two or three drops in a watch-glass being sufficient. After the sections have been in the stain fifteen minutes, the medullated fibres are found to be of a dark violet or blue, while the other elements are of a light yellow or yellowish red. If the sections are allowed to remain longer, from eighteen to twenty-four hours, an intense differential stain results.

¹ Edited by C. O. Whitman, Clark University, Worcester, Mass.

² *Anat. Anzeig.* IV., No. 7, 1889, p. 223.

When the desired grade of staining is reached, the sections are washed in alcohol, and mounted in Canada balsam.

Still finer results may be obtained if the sections, after lying some time in the stain, are placed in a saturate solution of sodium or lithium carbonate, for twenty-four hours.

The following method will give quite the same results as the above, and is used more easily.

Acetic acid (2 per cent. solution) . . . 100 ccm.
Hæmatoxylin (dissolved in alcohol) . . . 1 g.

A Simple Method for Removing the Gelatinous Layer from the Batrachian Egg.—Professor Blochmann³ recommends the following as a quick and safe method for treating large masses of frog eggs:

The spawn, having first been hardened in chrom-osmic-acetic acid, and then thoroughly washed in water, is placed in a beaker partly filled with dilute Eau de Tavelle (from three to four parts water), and gently agitated, that the fluid may reach all parts. In from fifteen to twenty minutes, according to the strength of the fluid, the glairy layer and the underlying membrane are dissolved, and the free eggs rest upon the bottom of the vessel.

At this point special care must be taken lest the eggs become injured by striking each other. The fluid being poured off, the eggs are carefully washed with water and placed through successive grades of alcohol.

If the material thus prepared is not made use of at once it should be kept in a dark place to prevent the further withdrawing of the chromic acid.

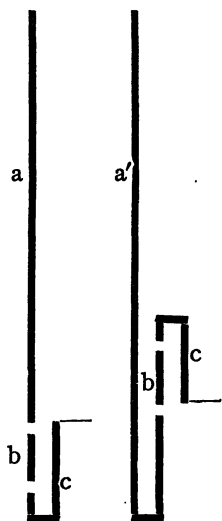
Eggs prepared in the above method stain nicely in borax carmine.

This method of Blochmann's is the same as that recommended by Whitman in June, 1889 (*vide* this journal).

The Differentiator Modified from report read before the British Association, September 11, 1889, at Newcastle, Eng.—According to the nature of the fluids in use, the differentiator takes one of the two forms here illustrated. As will be seen, a or a', the reservoir, b, the object-cylinder or object-box, and c, the filter, are three pieces of glass tubing joined together by means of caoutchouc tubing, the lat-

³ *Zoolog. Anz.* XII., No. 307, May, 1889, p. 269.

ter for the sake of clearness *not* shown. The filter, c, is easily made a follows: take a piece of glass tubing twice the length of the required filter, heat it red hot, draw it out to arm's length and break it in two; remove all the capillary part except about three inches on each half, heat, and bend into the required form (c); next carefully heat the capillary portion in a small alcohol flame, draw out exceedingly fine, and break off so as to leave a minute orifice.



To use the differentiator, proceed as follows: Suppose objects fixed by corrosive sublimate are to be studied in balsam after staining with borax-carmin. Fill the filter with sublimate solution and insert a plug of cotton (previously boiled in water to remove the air) at the u-bend. Join the object-box to the filter, fill up with sublimate solution, and push a plug of cotton into the lower end of the box, avoiding

bubbles. Wrap the cotton in fine linen if the subjects are minute. Put the objects into the box, plug the upper end in the same manner as the lower end, and finally join the box and filter thus filled to the empty reservoir a, and hang the instrument up in the position here illustrated. The objects are now transferred to 33 per cent. alcohol in the following manner: mix equal parts of sublimate solution and 33 per cent. alcohol (call this mixture 2). Mix equal parts of 2 and sublimate solution (call this 1). Mix equal parts of 2 and 33 per cent. alcohol (call this 3). Add mixture 1 to the reservoir until it is one-fourth full, mixture 2 until it is half full, mixture 3 until it is three-fourths full, and then fill up with 33 per cent. alcohol. If the successive mixtures are added with sufficient care, they will, owing to difference in specific gravity, remain distinct. If forced rapidly in, a nearly uniform mixture of about equal parts sublimate solution and 33 per cent. alcohol will result. The desirable procedure lies between these two suppositions, and gives rise to a uniform gradation of *differentiation from sublimate solution to 33 per cent. alcohol in passing upwards through the reservoir*. The flow, which at once commences drop-wise from the point of the filter, should be so regulated, either by tipping the instrument, or by breaking off more or less of the capillary part of the filter, as to cause the reservoir to be emptied in from two to five hours, when the objects will of course be in 33 per cent. alcohol, hav-

ing been *guarded to the utmost* against diffusion currents. They are now to be transferred to borax-carminé, a fluid *heavier* than 33 per cent. alcohol, by the use of reservoir a'. Fill the long arm of the reservoir with borax-carminé, and cork it, leaving the short arm empty and open. Mix equal parts of carminé and 33 per cent. alcohol (call this mixture 2). Mix equal parts of 2 and carminé (call this 1). Mix equal parts of 2 and 33 per cent. alcohol (call this 3). Add mixture 9 to the short arm of the reservoir until it is one-fourth full, mixture 2 until it is half full, mixture 3 until it is three-fourths full, and fill up with 33 per cent. alcohol. Transfer the object-box and filter to a', avoiding bubbles, and uncork the long arm. The flow begins as before, but this time *upward* through the object-box, and the object-box, and the objects are thus transferred in from two to five hours to borax-carminé.

After staining, the objects are transferred to 30 per cent. alcohol by means of reservoir a, the flow being so regulated that the change takes place in from five to ten hours. Then change successively to acidulated 70 per cent., 90 per cent., and absolute alcohol, allowing five, or better, ten hours to each change. Transfer to turpentine, chloroform, oil of cloves, or any oil desired by reservoir a'. Finally to thin balsam, *still by means of the differentiator*.

Whenever the objects are to be transferred to a lighter fluid, use reservoir a; whenever they are to be transferred to a heavier fluid, use reservoir a'. If objects are to be transferred to glycerine, transfer first to 50 per cent. glycerine in twelve hours, and then to pure glycerine in twenty-four hours.

Objects which defeat successful fixation by untimely contractions may be rendered insensible by means of the differentiator, and then fixed *perfectly outstretched*. Transfer them to alcohol of from 5 to 30 per cent. (or other paralyzing solution such as chloral hydrate) in from two to three hours, when they will be insensible and outstretched, and may be fixed as desired. A specially large object-box is easily contrived for larger specimens.

The differentiator, already in extensive use, was recently invented at Naples to overcome shrinkage and contraction in Anguillulidæ, and has speedily recommended itself for a great variety of delicate organisms, such as diatoms, desmids, and other delicate algæ, moulds, pteropods, and all sorts of embryological material, and takes its place at once as a permanent and very valuable addition to the paraphernalia of the biologist.